# IMMUNIZATION WITH GROUP A STREPTOCOCCAL CARBOHYDRATE PROTECTS AGAINST GROUP A STREPTOCOCCAL INFECTIONS IN MICE

Sabharwal H<sup>1</sup>, Blake M<sup>2</sup>, Michon F<sup>2</sup>, Zabriskie JB<sup>1</sup>.

<sup>1</sup>Laboratory of Clinical Microbiology and Immunology, The Rockefeller University, New York, USA, <sup>2</sup>North American Vaccine, Colombia, USA

### **Abstract**

Our previous studies have demonstrated that rabbit sera raised against Group A Streptococcal carbohydrate could protect mice against a lethal challenge with two different M+ type specific Group A Streptococcal strains. The vast majority of the control mice died when compared to the mice protected by the antibody (p<0.001).

To further investigate the role of Group A carbohydrate antibodies for active protection, mice were immunized with two different preparations of Group A carbohydrate and the mortality was monitored and compared with a group of mice immunized with just tetanus toxoid and adjuvant. The mice were then challenged with two distinct M+ type specific strains and the mortality monitored. The immunized mice were protected against the lethal challenge with the streptococcal strains compared with the control animals (p = 0.003).

The Group A carbohydrate antibodies were tested for cross reactivity with human kidney, heart and liver tissue sections by immuno-fluoroescence. The antibodies did not react with these tissues indicating that the Group A carbohydrate antigen can be considered a safe candidate for a vaccine against Group A Streptococcal infections.

## Introduction

Previous studies from our laboratory have demonstrated that Streptococcal Group A carbohydrate (CHO) antibodies are present in the sera of normal children and increase in titer with age<sup>1</sup>. Using an in vitro phagocytic assay employing human mononuclear cells, these antibodies were shown to be opsonic for several distinct Type specific M+ streptococcal strains. To determine whether antibodies to the Group A CHO raised in rabbits could also phagocytose Group A streptococci in the in vitro assay, the sera of immunized rabbits were introduced into the opsonic assay. The sera of these rabbits also were opsonic for two different M+ strains<sup>1</sup>.

In view of these results, experiments were designed to determine whether Group A antibodies raised in rabbits could passively protect against a lethal challenge in mice employing two different M+ strains. The second set of experiments were designed to determine whether active immunization of mice using the immunogen coupled to adjuvants commonly used in humans could protect against a similar lethal challenge model.

The present report demonstrates that both passive immunization with rabbit anti-carbohydrate antibodies and active immunization with Group A CHO conjugated to Tetanus Toxoid plus alum protects against a lethal Group A streptococcal infection in mice.

## Materials and Methods

Bacterial Strains: All streptococcal strains were obtained from the Rockefeller University Collection. The following strains were used: S43/46 (M Type 6), D58/93/7 (M Type 3), S23 (M Type 14).

Challenge Assay: The strain to be tested was suspended in 5 ml of Todd-Hewitt broth and grown for 18 hours at 37°C. The overnight culture was diluted 1:3 with fresh pre-warmed TH broth and incubated for approximately 2 hrs at 37°C or until the OD reached a 0.73 reading at 600 nm. The culture was then appropriately diluted to deliver sufficient numbers of Group A streptococci to

cause 100 % mortality in the control mice over a 72 hour time period. Injections were given IP in a volume of 200  $\mu$ l. 20 and 100  $\mu$ l samples of the same dilutions were placed in petri dishs to which 10 ml of Brain heart infusion agar (2.4%) containing 0.5% defribinated sheep blood was added. The plates were incubated overnight at 37°C and the colonies counted the next day.

Immunizations: A. <u>Passive</u>: Rabbits were immunized with Group A CHO conjugated to tetanus toxoid at a dose of 500ugs in 4 separate sites in Complete Freund's adjuvant. The second dose was given 3 weeks later in incomplete Freund's. Animals were bled 2 weeks later and the CHO antibody titers assayed. Animals were boosted subcutaneously with the same dose mixed with incomplete adjuvant until titers of  $1 \times 10^6$  were reached. On the day of the murine challenge, 0.3mL of the rabbit antibody (usually diluted 1:5 or 1:10) or normal rabbit serum was injected IP 1 hr before challenge.

B. Active: Mice were injected subcutaneously with Group A carbohydrate conjugated to TT at a concentration of 5  $\mu$ g (200  $\mu$ l) per dose mixed with alum. The second dose was given 4 weeks later and the procedure was repeated twice for a total of four doses. Animals were checked for CHO titers by retro-orbital bleeding 10 days after the last dose. Control mice were injected in the same manner with adjuvant and TT alone without the addition of carbohydrate. Animals were challenged IP with live Group A streptococci as described above.

## Results

Passive Protection Studies: To determine whether pure Group A carbohydrate antibodies could protect against a lethal challenge model with live Group A streptococci, mice were challenged with two different M+ type specific strains IP. The dose was regulated so that a majority of the control mice died within 72 hours after the injection. As seen in Tables 1 and 2, the rabbit anti CHO antibody was able to passively protect animals irrespective of the type of Group A Streptococcal strain used for the challenge. While the amount of colonies administered IP did differ depending on the strain used, the vast majority of the control mice died when compared to the mice protected by the antibody.

Table 1 and Table 2

Active Immunization Studies: The challenge experiments described above were repeated in the actively immunized mice. Again two distinct M+ type specific strains were employed. Tables 3 and 4 demonstrate that immunization of mice with carbohydrate preparation coupled to TT and alum adjuvant markedly protected against a lethal challenge model of live streptococci irrespective of the M+ type. Control animals immunized with just the TT and conjugate with alum without the carbohydrate did not protect against the lethal challenge.

Table 3 and Table 4

#### Discussion

Our previous work clearly demonstrated that human sera contain antibodies to Group A streptococcal carbohydrate and these titers increase with age<sup>1</sup>. Furthermore these antibodies are opsonic in an in vitro phagocytosis assay and were able to phagocytose several different M+ type specific Group A streptococcal strains.

Based on these results, we next asked the question of whether either by passive administration of Group A CHO antibody or active immunization of animals we could protect mice against a lethal challenge model of IP administration of live Group A streptococci. The answer to both questions was definitely affirmative. Rabbit antibodies raised against pure Group A carbohydrate clearly protected against a lethal challenge with two M+ type specific streptococcal strains. Almost

identical results were obtained in the active immunization studies where immunization with Group A CHO conjugated to TT also protected the animals.

These results strongly support the concept that Group A streptococcal carbohydrate can be used as a vaccine to prevent Group A streptococcal infections irrespective of the M+ type specific strain.

## References

1. Salvadori LG, Blake MS, McCarty M, Tai JY, Zabriskie JB. Group A Streptococcusliposome ELISA antibody titers to Group A polysaccharide and opsonophagocytic capabilities of the antibodies. J Infect Dis, 1995; 171:593-600.

Table 1. Passive protection test in mice against Group A Streptococcus Type 6 (S43/46).

Serum	Colonies	Mice*
NRS	200-500	3/26**
Group A CHO Ab	200-500	16/26

<sup>\*</sup>Number of Mice Survived/Injected

Table 2. Passive protection test in mice against Group A Streptococcus Type 3 (D58/93/7).

Serum	Colonies	Mice*
NRS	1.7-4.6 X 10 <sup>5</sup>	3/15**
Group A CHO Ab	1.7-4.6 X 10 <sup>5</sup>	13/15

<sup>\*</sup>Number of Mice Survived/Injected

Table 3. Active immunization studies with Group A Streptococcal CHO in mice challenged with

Type 6 (S43/46) Streptoco Group	Adjuvant	Inoculum Range	Survived/Injected
CHO-TT Conjugate	Alum	3-7 X 10 <sup>5</sup>	11/15*
TT	Alum	3-7 X 10 <sup>5</sup>	2/15*

Table 4. Active immunization studies with Group A Streptococcal CHO in mice challenged with live type 14 (S23) Streptococci.

live type 14 (S23) Streptoc Group	Adjuvant	Inoculum Range	Survived/Injected
CHO-TT Conjugate	Alum	3-3.6 X 10 <sup>6</sup>	18/23*
TT	Alum	3-3.6 X 10 <sup>6</sup>	5/22*

<sup>\*</sup>p<0.001

<sup>\*\*</sup>p<0.001

<sup>\*\*</sup>p<0.041